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SIMULTANEOUS MEASUREMENT OF MILK INTAKE AND TOTAL ENERGY EXPENDITURE IN MIXED-FED INFANTS: METHODOLOGICAL APPROACH AND PREDICTION OF TOTAL BODY WATER

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Abstract

Evaluation of the energy metabolism that underlies the new WHO breast-fed growth reference requires simultaneous measurements of milk volume intake (MVI) and total energy expenditure (TEE) by stable isotope methodologies. In young infants, such data is collected without difficulty using the dose-to-the-infant method. In older infants, where breast-milk is supplemented with non-milk foods, MVI must be measured by dosing the mother instead of the infant. This procedure would interfere with a simple measurement of infant TEE using the standard dose-to-the-infant method. Theoretically, this difficulty can be resolved by dosing the mother with deuterium and the infant with 18-oxygen, and using curve-peeling methods to calculate the infant deuterium kinetics. We propose to ascertain whether such an approach is viable in practice, such that MVI, TEE and body composition could all be measured simultaneously in mixed-fed infants. Where MVI in older infants is measured on its own, there is a need to predict infant body water in order to estimate the deuterium dilution space. Using a database of 234 infants aged 1.5 to 12 months, we provide new predictive equations by which such values may be obtained.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Up until the mid 1980's, recommendations for energy requirements of infants and children were based on observations of food intakes in healthy subjects. However food intakes need not necessarily reflect food requirements, and the 1985 WHO/FAO/UNU report proposed a more appropriate approach whereby requirements would be based on measurements of total energy expenditure, with an added component to account for the energy stored in tissue deposited during growth [1]. Following this report, a number of studies measured total energy expenditure in young infants, their results recently being summarized by Butte [2].

This research on early infant energy metabolism further demonstrated differences in energy utilization between formula-fed and breast-fed infants, who have also been observed to grow at different rates in some populations [3,4] though not in all cases [5,6].

Since populations worldwide vary in the prevalence of breast-feeding, with developing country populations tending to show much higher rates than industrialized populations, it is apparent that reference data for both growth and energy requirements must take into account feeding mode. The existing growth standards formally adopted by WHO, the NCHS data set, were collected in the United States at a time when formula feeding was widespread. The WHO has therefore undertaken to collect new reference data, focusing on exclusively breast-fed infants, which are more appropriate for widespread use in developing countries. In order to update energy requirements, it is therefore necessary to collect data on the energy metabolism that underlies the growth patterns of such breast-fed infants.

Total energy expenditure (TEE), body composition (BC) and milk volume intake (MVI) can all be measured in free-living infants using stable isotope methodologies in early life. Deuterium is required for all three measurements, while 18-oxygen contributes to the TEE measurement, and duplicates the BC measurement. Each measurement of infant TEE automatically provides isotope dilution spaces from which BC can be calculated. Furthermore, in exclusively milk-fed infants, where all oral water intake is obtained from milk, MVI can also be calculated. Thus, in young exclusively milk-fed infants all three measurements can be obtained simultaneously from a single *dose-to-the-infant* procedure [7,8,9].

In older infants, where milk is supplemented by other foods, a single dosing of the infant can measure TEE and BC but not MVI. This represents no problem in formula-fed infants, where MVI is measured readily by weighing formula bottles before and after feeds. However, breast-milk intake would need to be measured by test weighing. This procedure is cumbersome, intrudes on the mother's time, and is inappropriate for many field studies. MVI in mixed-fed infants can nevertheless be measured using stable isotope methodologies, using the *dose-to-the-mother* method whereby deuterium is given to the mother, and its appearance in the infant used to infer breast-milk intake [10].

Although both dose-to-the-infant and dose-to-the-mother methods have both been used with great success, the dose-to-the-mother MVI method currently suffers from limitations. Firstly, since deuterium is given to the mother, a further dose of deuterium to the infant to measure TEE and BC would complicate the MVI measurement. Second, the dose-to-the-mother method requires that the infant isotope dilution space (effectively total body water) be measured by a separate infant dose of 18-oxygen, or be predicted from anthropometry. Currently there are no such equations suitable for older infants.

In this proposal, we therefore seek to address these issues. Our specific aims are:

a) To determine whether TEE, BC and MVI can be measured simultaneously in older infants by dosing deuterium to the mother and ^{18}O to the infant.

b) To derive new equations for the prediction of infant total body water from anthropometry, using an existing data set comprising 234 infants aged 6 weeks to 12 months

To accomplish these aims we will:

a) Collect data on isotope turnover in 10 mother-infant dyads where the infant is mixed fed, and use curve-peeling models to infer both breast-milk intake and water turnover in the infant from a single maternal dosing of deuterium.

b) Undertake multiple regression analysis, with total body water as the dependent variable and weight, length, sex, age, triceps skin fold, sub scapular skin fold and feeding mode as potential independent variables.

2. METHODS

2.1. Stable isotope kinetics in infant and mother

In the *dose-to-the-infant* method, each isotope equilibrates with the body water pool, and also exchanges to a small extent with non-aqueous hydrogen and oxygen. For deuterium, the extent of this exchange varies with infant age due to the effects of body composition. Thus if only MVI or BC is being measured using deuterium alone, age-specific correction factors are required to convert the dilution space (N_D) into total body water (TBW) [11]. Following equilibration, the washout kinetics of each isotope can be ascertained from daily urine samples, allowing the rate constants (k_D and k_O) to be determined. Since infants are fed frequently, the dose of tracer given to the infant will be diluted during the equilibration period. This artifact is addressed in both isotopes by using the value of k to calculate an assumed value for N at the time of dosing [12,13].

In the *dose-to-the-mother* method for calculating MVI, maternal and infant isotope kinetics in combination allows calculation of an infant maternal-k_D value, i.e. water intake from the mother. However, because the infant is not dosed directly, N_D cannot be calculated from the deuterium data, and must be obtained by a separate measurement technique or predicted. Nor can the total infant k_D value be obtained from a simple transformation of the deuterium data.

These difficulties could be resolved as follows. Firstly, an additional infant dosing of 18-oxygen would provide both k_O and N_D values, allowing N_D to be calculated from N_O using the age-specific correction factors described above [11]. Secondly, the application of curve-peeling procedures to the deuterium data would distinguish water intake both from the mother and from the environment in total. In this situation, therefore, all the data required for calculation of MVI, BC and TEE would be available at a single time-point in the mixed-fed infant.

We are currently undertaking such a study where infant MVI and TEE will be measured simultaneously through dual dosing of infants and mothers. The infants will be mixed-fed and distributed evenly over the age range 4 – 12 months post-partum. The mother will receive 10 g D_2O. Due to the current severe shortage, infant ^{18}O data will be simulated from our existing infant database rather than obtained from an actual dosing. Maternal saliva and infant urine samples will be collected daily over a 14-day period, enabling the isotope kinetics to be determined.

Curve peeling will be used to calculate k_D from the non-linear changes in deuterium concentration in the infant body water pool.

2.2. The relationship between TBW and anthropometry, age and sex in infancy

In the absence of this additional dosing, the value for N_D can be obtained by predicting TBW using simple anthropometric variables, age and sex. Such an approach requires data covering the entire period of infancy.

Data on TBW have been collected in a number of studies of TEE during the late 1980's and early 1990's. The data comprise 234 measurements performed on 124 infants aged 6 weeks to 12 months post-partum, with 37 infants being measured on more than one occasion. Data on total energy expenditure in this sample have been described previously, and summarized as a single dataset [14]. In each case, TBW was derived from the 18-oxygen dilution space, using the back extrapolation method [13]. Characteristics of the sample are given in Table I.

Multiple regression analysis was used to derive prediction equations for total body water for the entire sample of infants, and for breast-fed and formula-fed infants separately. Potential predictive variables included age, sex, weight, length, triceps and sub scapular skin folds, 7-day weight gain and feeding mode. Stepwise regression analysis was first used to identify significant predictors. Equations were then derived using LogTBW as the dependent variable. Weight and skin folds were also expressed in natural logarithmic form, other variables were not transformed. This approach allows the standard error of the regression to be expressed as a percentage of the predicted value.

3. RESULTS

Data from the dual dosing study are currently being collected.

Regression equation details for the entire sample are given in Table II. TBW was predicted with a standard error of 6.9 % using the best model, in which the independent variables were weight, sub scapular skin fold, sex and age. A model using weight, sex and age gave a standard error of 7.3 %, while weight alone gave a standard error of 7.6 %.

Diet was not a significant predictor in these models, nor did its contribution improve if the analysis was restricted to the infants aged <6 months, in whom the effects of supplementary foods were minimal. We nevertheless investigated the relationships separately in the diet groups, given the population differences in growth reported previously. Equations for each diet group are given in Table III. For breast-fed infants, the best model comprised weight and sub scapular skin fold, giving a standard error of 7.9 %. An alternative model using weight, sex and age had a standard error of 8.2 %, while weight alone gave a standard error of 8.5 %. For formula-fed infants, the best-fit model used weight, sex and age, giving a standard error of 5.7%. Alternative models either using weight and sub scapular skin fold, or weight alone, had standard errors of 6.5 %.

Separate equations for the sexes were also derived, given in Table IV. The best models including age and weight gave standard error values of 6.5 % (males) and 7.3 % (females), but the model using weight alone was only marginally poorer, with standard errors of 6.9 % (males) and 7.8 % (females).

No significant improvement in the standard error values were obtained in comparison to the entire sample, and the regression coefficients were very similar between the sexes.

Our analysis therefore indicates that infant TBW can be predicted with an error of around 7 %, using anthropometric indices, age and sex as predictive variables. A range of predictive equations is presented, thus potentially increasing the contexts in which TBW can be predicted. Weight is reasonably successful as the sole predictor, while the addition of additional anthropometric information or sex improves the model marginally. Where age is known, it is a better predictive variable than length, probably because length is difficult to measure with high precision in infants.

If the entire first year of infancy is considered, there is little effect of feeding mode in this population. Even if the infants aged 6 months and older are excluded, such that the remaining subjects are infants almost exclusively breast-fed or formula-fed, no significant effect of diet on body composition was found. We have commented previously that our study population is not representative of the national population, in that our breast-feeding and formula-feeding mothers are of similar educational level and socio-economic status. However, where statistically significant average differences between the diet groups in anthropometry are reported, they remain small in magnitude. Variability within each diet group is far greater than that between the groups, and use of our equations is unlikely to introduce serious bias when applied to healthy breast-fed or formula-fed infants.

There have been two previous evaluations of the relationship between TBW and anthropometry in infancy. The classic paper from 1957 by Friis-Hansen [15] included 36 infants aged <1 year, but from the individual data provided in this report it can be seen that the infants are on average a standard deviation score lower ($p<0.001$) in weight compared to contemporary UK children [16] (see Fig 1). Thus the relationship between TBW and anthropometry is expected to have changed during the last 4 decades due to improved nutrition and health. A more recent study by Butte and colleagues [17] considered the same relationship in 40 infants aged 1 or 4 months. The resulting equations may not necessarily be valid in older infants, given that the proportion of weight that is fat changes markedly during the first year of life [18].

Our study demonstrates a relationship between weight and TBW that differs from those in both previous studies, as is shown in Figure 2. Comparison of our study and that of Friis-Hansen indicates a consistently greater amount of weight per kg of body water in Cambridge infants, confirming that contemporary infants are significantly heavier (see Fig 1) and therefore fatter. The equations of Butte et al. were derived from younger infants, and are not relevant to older infants. This influences not only their slope but also their intercept, as our equations incorporate infants in the middle of the infancy period who tend to be fatter. Thus again, our equations indicate a trend to greater weight for a given value of TBW compared to Butte et al., and the latter equations may be more suitable for younger infants.

4. PLANS FOR FUTURE WORK

If our proposed dual dosing method proves successful in measuring TEE and MVI successfully in mixed-fed infants, then we propose to apply the method in developing country populations, for example in Pelotas, Brazil where information on such variables is required. The method will aid in resolving the current problem that the great majority of infant energy metabolism data obtained using stable isotope technologies relates to the first few months post-partum, while older infants have been relatively ignored.

Our equations for the prediction of TBW have wider potential application beyond their role in the dose-to-the-mother method of MVI estimation. Infant TBW and hence fat-free mass may be predicted from anthropometry in community studies in order to assess growth status in general. However their accuracy will be influenced by the degree of body fatness, so in developing country populations they are predicted to work better in stunted than in wasted infants.

Legends for illustration
Figure 1. Weight Z-score in Friis-Hansen's infants [15] relative to contemporary UK reference data.

Figure 2. Relationship between weight and total body water in the studies of Friis-Hansen [15], Butte et al. [17] and the present study.

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TABLE I. CHARACTERISTICS OF THE SAMPLE

	6 weeks Mean	SD	3 months Mean	SD	6 months Mean	SD	9 months Mean	SD	12 months Mean	SD
Number	49		92		37		36		18	
Age (days)	36	3	81	6	181	5	276	4	368	5
Weight (kg)	4.54	0.48	5.89	0.60	7.70	0.77	8.91	1.14	10.27	1.36
Height (cm)	54.7	1.8	59.9	2.1	66.7	2.1	71.6	2.9	76.8	3.7
Triceps (mm)	6.6	0.9	7.7	1.3	7.6	1.3	8.3	2.2	9.0	2.1
Subscap (mm)	6.8	1.0	7.6	1.4	7.3	1.3	6.6	1.3	6.6	1.7
% breast-fed	41		42		51		58		83	
% male	41		44		35		34		55	
TBW (litres)	2.89	0.27	3.51	0.37	4.37	0.37	5.11	0.68	6.04	0.78

TABLE II. REGRESSION STATISTICS FOR LOGTBW FOR THE ENTIRE SAMPLE OF 234 INFANTS

	Coefficient	Coefficient error	Standard error	R^2
Constant	-0.180	0.032	0.076	90.9
LogWT	0.824	0.017		
Constant	0.157	0.060	0.070	92.3
LogWT	0.640	0.036		
Age	0.0005	0.0001		
Sex	-0.046	0.009		
Constant	0.237	0.065	0.069	92.6
LogWT	0.693	0.040		
Age	0.0004	0.0001		
Sex	-0.046	0.009		
LogSUBS	-0.080	0.027		

Where TBW = total body water in litres, WT = weight in kg, SUBS = sub scapular skin fold in mm, age in days, sex: m=1, f=2, using natural logarithms.
Equations are read from the table as shown below for the first example:
LogTBW = -0.180 + (0.824 * logWT)

TABLE III. REGRESSION STATISTICS FOR LOGTBW FOR BREAST-FED AND FORMULA-FED INFANTS

	Coefficient	Coefficient error	Standard error	R^2
Breast-fed (n=117)				
Constant	-0.235	0.053	0.085	89.4
LogWT	0.854	0.027		
Constant	0.124	0.094	0.079	90.9
LogWT	0.853	0.026		
LogSUBS	-0.186	0.041		
Constant	0.113	0.114	0.082	90.7
LogWT	0.655	0.066		
Age	0.0005	0.0002		
Sex	-0.033	0.016		
Formula-fed (n=117)				
Constant	-0.120	0.039	0.065	92.2
LogWT	0.790	0.021		
Constant	-0.023	0.066	0.065	92.5
LogWT	0.796	0.021		
LogSUBS	-0.055	0.030		
Constant	0.179	0.064	0.057	94.3
LogWT	0.637	0.040		
Age	0.0005	.0001		
Sex	-0.057	0.011		

Where TBW = total body water in litres, WT = weight in kg, SUBS = sub scapular skin fold in mm, age in days, sex: m=1, f=2, using natural logarithms.

TABLE IV. REGRESSION STATISTICS FOR LOGTBW FOR MALE AND FEMALE INFANTS.

	Coefficient	Coefficient error	Standard error	R^2
Males (n = 96)				
Constant	-0.157	0.044	0.069	93.0
LogWT	0.823	0.023		
Constant	0.093	0.083	0.065	93.8
LogWT	0.651	0.054		
Age	0.0005	0.0001		
Females (n = 136)				
Constant	-0.176	0.044	0.078	89.5
LogWT	0.814	0.024		
Constant	0.078	0.073	0.073	90.2
LogWT	0.632	.049		
Age	0.0005	0.0001		

Where TBW = total body water in litres, WT = weight in kg, SUBS = sub scapular skin fold in mm, age in days, sex: m=1, f=2, using natural logarithms.

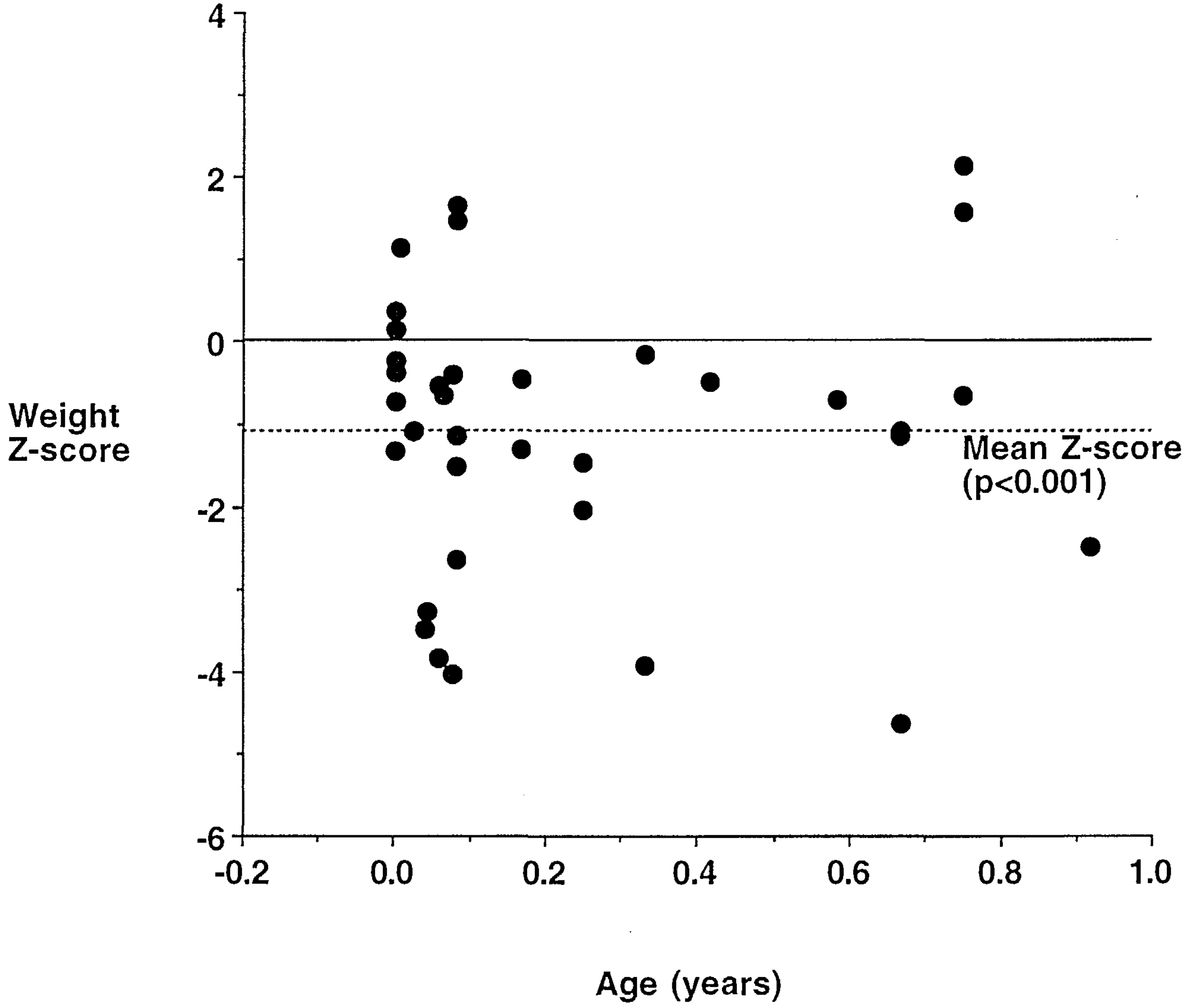

Fig. 1. Weight Z-score in Friis-Hansen's infants [15] relative to contemporary UK reference data.

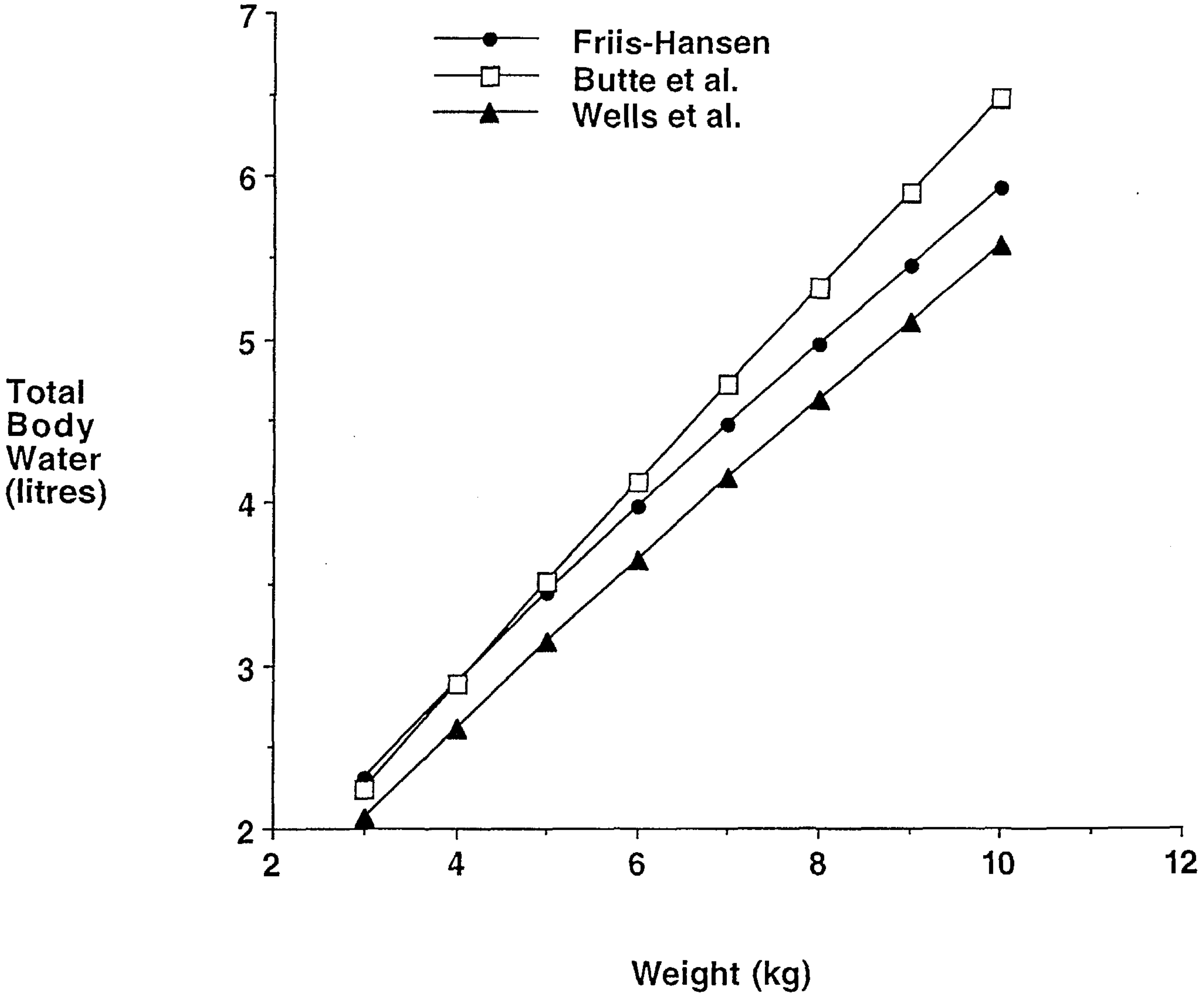

Fig. 2. Relationship between weight and total body water in the studies of Friis-Hansen [15], Butte et al. [17] and the present study.